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Leaf segmentation in plant phenotyping: a collation study

Hanno Scharr¹ · Massimo Minervini² · Andrew P. French³ · Christian Klukas⁹ ·
David M. Kramer⁵ · Xiaoming Liu⁶ · Imanol Luengo³ ·
Jean-Michel Pape⁴ · Gerrit Polder⁷ · Danijela Vukadinovic⁷ · Xi Yin⁶ ·
Sotirios A. Tsafaris^{2,8}

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Abstract Image-based plant phenotyping is a growing application area of computer vision in agriculture. A key task is the segmentation of all individual leaves in images. Here we focus on the most common rosette model plants, Arabidopsis and young tobacco. Although leaves do share

appearance and shape characteristics, the presence of occlusions and variability in leaf shape and pose, as well as imaging conditions, render this problem challenging. The aim of this paper is to compare several leaf segmentation solutions on a unique and first-of-its-kind dataset containing images from typical phenotyping experiments. In particular, we report and discuss methods and findings of a collection of submissions for the first Leaf Segmentation Challenge of the Computer Vision Problems in Plant Phenotyping workshop in 2014. Four methods are presented: three segment

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✉ Massimo Minervini
m.minervini@imtlucca.it

✉ Sotirios A. Tsafaris
stsajt@gmail.com

Hanno Scharr
h.scharr@fz-juelich.de

Andrew P. French
Andrew.P.French@nottingham.ac.uk

Christian Klukas
christian.klukas@lemnatec.de

David M. Kramer
kramerd8@cns.msu.edu

Xiaoming Liu
liux@cse.msu.edu

Imanol Luengo
psxill@nottingham.ac.uk

Jean-Michel Pape
pape@ipk-gatersleben.de

Gerrit Polder
gerrit.polder@wur.nl

Danijela Vukadinovic
danijela.vukadinovic@wur.nl

Xi Yin
yinxil@cse.msu.edu

¹ Institute of Bio- and Geosciences: Plant Sciences (IBG-2)
Forschungszentrum Jülich GmbH, Jülich, Germany

² IMT Institute for Advanced Studies, Lucca, Italy

³ Schools of Biosciences and Computer Science, Centre for
Plant Integrative Biology, University of Nottingham,
Nottingham, UK

⁴ Department of Molecular Genetics, Leibniz Institute of Plant
Genetics and Crop Plant Research (IPK), Gatersleben,
Germany

⁵ Departments of Energy Plant Research Lab, and
Biochemistry and Molecular Biology, Michigan State
University, East Lansing, MI, USA

⁶ Department of Computer Science and Engineering, Michigan
State University, East Lansing, MI, USA

⁷ Greenhouse Horticulture, Wageningen University and
Research Centre, Wageningen, Netherlands

⁸ School of Engineering, University of Edinburgh, Edinburgh,
UK

⁹ LemnaTec GmbH, Aachen, Germany

leaves by processing the distance transform in an unsupervised fashion, and the other via optimal template selection and Chamfer matching. Overall, we find that although separating plant from background can be accomplished with satisfactory accuracy ($>90\%$ Dice score), individual leaf segmentation and counting remain challenging when leaves overlap. Additionally, accuracy is lower for younger leaves. We find also that variability in datasets does affect outcomes. Our findings motivate further investigations and development of specialized algorithms for this particular application, and that challenges of this form are ideally suited for advancing the state of the art. Data are publicly available (online at <http://www.plant-phenotyping.org/datasets>) to support future challenges beyond segmentation within this application domain.

Keywords Plant phenotyping · Leaf · Multi-instance segmentation · Machine learning

1 Introduction

The study of a plant's phenotype, i.e. its performance and appearance, in relation to different environmental conditions is central to understanding plant function. Identifying and evaluating phenotypes of different cultivars (or mutants) of the same plant species, are relevant to, e.g. seed production and plant breeders. One of the most sought-after traits is plant growth, i.e. a change in mass, which directly relates to yield. Biologists grow model plants, such as *Arabidopsis* (*Arabidopsis thaliana*) and tobacco (*Nicotiana tabacum*), in controlled environments and monitor and record their phenotype to investigate general plant performance. While previously such phenotypes were annotated manually by experts, recently image-based nondestructive approaches are gaining attention among plant researchers to measure and study visual phenotypes of plants [23, 26, 39, 54].

In fact, most experts now agree that lack of reliable and automated algorithms to extract fine-grained information from these vast datasets forms a new bottleneck in our understanding of plant biology and function [37]. We must accelerate the development and deployment of such computer vision algorithms, since according to the Food and Agriculture Organization of the United Nations (FAO), large-scale experiments in plant phenotyping are a key factor in meeting agricultural needs of the future, one of which is increasing crop yield for feeding 11 billion people by 2050.

Yield is related to plant mass and the current gold standard for measuring mass is weighing the plant; however, this is invasive and destructive. Several specialized algorithms have been developed to measure whole-plant properties and particularly plant size [6, 17, 24, 29, 38, 52, 54, 60]. Nondestructive measurement of a plant's projected leaf area (PLA), i.e. the

counting of plant pixels from top-view images, is considered a good approximation of plant size for rosette plants and is currently used. However, when studying growth, PLA reacts relatively weakly, as it includes growing and non-growing leaves, but the per-leaf-derived growth (implying a per-leaf segmentation), has a faster and clearer response. Thus, for example, growth regulation [5] and stress situations [26] can be evaluated in more detail. Additionally, since growth stages of a plant are usually based on the number of leaves [15], an estimate of leaf count as provided by leaf segmentation is beneficial.

However, obtaining such refined information at the individual leaf level (as for example in [55]) which could help us identify even more important plant traits is, from a computer vision perspective, particularly challenging. Plants are not static, but changing organisms with complexity in shape and appearance increasing over time. Over a period of hours, leaves move and grow, with the whole plant changing over days or even months, in which the surrounding environmental (as well as measurement) conditions may also vary.

Considering also the presence of occlusions, it is not surprising that the segmentation of leaves from single view images (a multi-instance image segmentation problem) remains a challenging problem even in the controlled imaging of model plants. Motivated by this, we organized the Leaf Segmentation Challenge (LSC) of the Computer Vision Problems in Plant Phenotyping (CVPPP 2014) workshop,¹ held in conjunction with the 13th European Conference on Computer Vision (ECCV), to assess the current state of the art.

This paper offers a collation study and analysis of several methods from the LSC challenge, and also from the literature. We briefly describe the annotated dataset, the first of its kind, that was used to test and evaluate the methods for the segmentation of individual leaves in image-based plant phenotyping experiments (see Fig. 1 and also [46]). Colour images in the dataset show top-down views on rosette plants. Two datasets show different cultivars of *Arabidopsis* (*A. thaliana*), while another one shows tobacco (*N. tabacum*) under different treatments. We manually annotated leaves in these images to provide ground truth segmentation and defined appropriate evaluation criteria. Several methods are briefly presented, and in the results section, we discuss and evaluate each method.

The remainder of this article is organized as follows: Sect. 2 offers a short literature review, while Sect. 3 defines the adopted evaluation criteria. Section 4 presents the datasets and annotations used to support the LSC challenge, which is described in Sect. 5. Section 6 describes the methods compared in this study, with their performance and results

¹ <http://www.plant-phenotyping.org/CVPPP2014>.

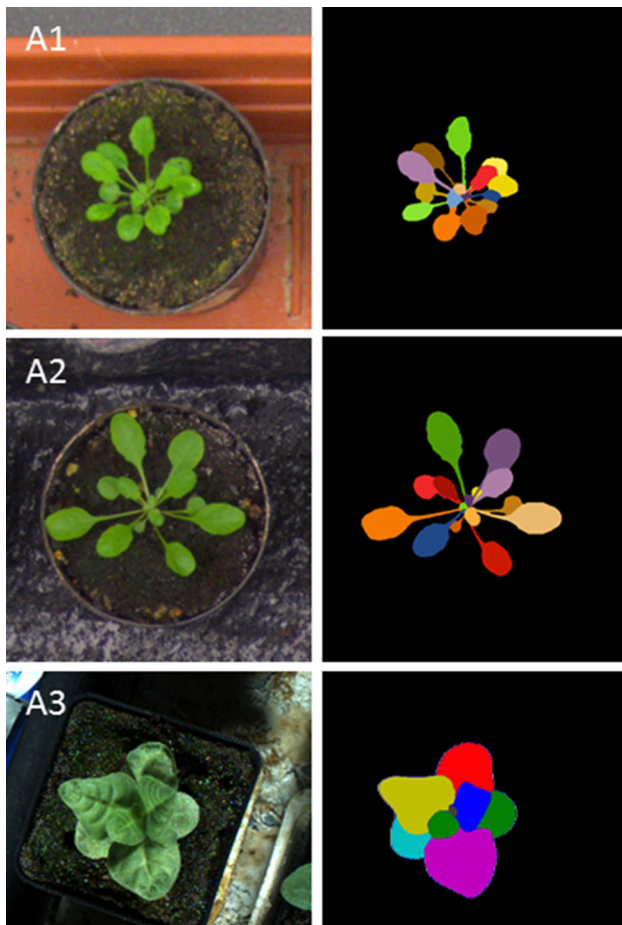


Fig. 1 Example images of Arabidopsis (A1, A2) and tobacco (A3) from the datasets used in this study [46]

discussed in Sect. 7. Finally, Sect. 8 offers conclusions and outlook.

2 Related work

At first glance, the problem of leaf segmentation appears similar to leaf identification and isolated leaf segmentation (see e.g. [12–14, 28, 48, 58]), although as we will see later it is not. Research on these areas has been motivated by several datasets showing leaves in isolation cut from plants and imaged individually, or showing leaves on the plant but with a leaf encompassing a large field of view (e.g. by imaging via a smart phone application). This problem has been addressed in an unsupervised [48, 58], shape-based [13, 14, 28], and interactive [12–14] fashion.

However, the problem at hand is radically different. The goal, as the illustrative example of Fig. 1 shows, is not to identify the plant species (usually known in this context) but to segment accurately each leaf in an image showing a whole plant. This multi-instance segmentation problem is excep-

tionally complex in the context of this application. This is not only due to the variability in shape, pose, and appearance of leaves, but also due to lack of clearly discernible boundaries among overlapping leaves with typical imaging conditions where a top-view fixed camera is used. Several authors have dealt with the segmentation of a live plant from background to measure growth using unsupervised [17, 24] and semi-supervised methods [34], but not of individual leaves. The use of colour in combination with depth images or multiple images for supervised or unsupervised plant segmentation is also common practice [4, 10, 27, 44, 47, 49, 50, 56].

Several authors have considered leaf segmentation in a tracking context, where temporal information is available. For example, Yin et al. [60] segment and track the leaves of Arabidopsis in fluorescence images using a Chamfer-derived energy functional to match available segmented leaf templates to unseen data. Dellen et al. [18] use temporal information in a graph-based formulation to segment and track leaves in a high spatial and temporal resolution sequence of tobacco plants. Aksoy et al. [3] track leaves over time, merging segments derived by superparametric clustering by exploiting angular stability of leaves. De Vyllder et al. [16] use an active contour formulation to segment and track Arabidopsis leaves in time-lapse fluorescence images.

Even in the general computer vision literature, this type of similar-appearance, multi-instance problem is not well explored. Although several interactive approaches exist [22, 40], user interaction inherently limits throughput. Therefore, here we discuss automated learning-based object segmentation approaches, which might be adaptable to leaf segmentation. Wu and Nevatia [57] present an approach that detects and segments multiple, partially occluded objects in images, relying on a learned, boosted whole-object segmentor and several part detectors. Given a new image, pixels showing part responses are extracted and a joint likelihood estimation inclusive of inter-object occlusion reasoning is maximized to obtain final segmentations. Notably, they test their approach on classical pedestrian datasets, where appearance and size variation does exist, so in leaf segmentation where neighbouring leaves are somewhat similar, this approach might yield less appealing results. Another interesting work [45] relies on Hough voting to jointly detect and segment objects. Interestingly, beyond pedestrian datasets they also use a dataset of house windows where appearance and scale variation is high (as is common also in leaves), but they do not overlap. Finally, graphical methods have also been applied to resolve and segment overlapping objects [25], and were tested also on datasets showing multiple horses.

Evidently, until now, the evaluation and development of leaf segmentation algorithms using a common reference dataset of individual images without temporal information is lacking, and therefore is the main focus of this paper.

3 Evaluation measures

Measuring multi-object segmentation accuracy is an active topic of research with several metrics previously proposed [31–33, 42]. For the challenge and this study, we adopted several evaluation criteria and devised Matlab implementations. Some of these metrics are based on the Dice score of binary segmentations:

$$\text{Dice (\%)} = \frac{2|P^{\text{gt}} \cap P^{\text{ar}}|}{|P^{\text{gt}}| + |P^{\text{ar}}|}, \quad (1)$$

measuring the degree of overlap among ground truth P^{gt} and algorithmic result P^{ar} binary segmentation masks.

Overall, two groups of criteria were used. To evaluate segmentation accuracy we used:

- Symmetric Best Dice (SBD), the symmetric average Dice among all objects (leaves), where for each input label the ground truth label yielding maximum Dice is used for averaging, to estimate average leaf segmentation accuracy. Best Dice (BD) is defined as

$$\text{BD}(L^a, L^b) = \frac{1}{M} \sum_{i=1}^M \max_{1 \leq j \leq N} \frac{2|L_i^a \cap L_j^b|}{|L_i^a| + |L_j^b|}, \quad (2)$$

where $|\cdot|$ denotes leaf area (number of pixels), and L_i^a for $1 \leq i \leq M$ and L_j^b for $1 \leq j \leq N$ are sets of leaf object segments belonging to leaf segmentations L^a and L^b , respectively. SBD between L^{gt} , the ground truth, and L^{ar} , the algorithmic result, is defined as

$$\text{SBD}(L^{\text{ar}}, L^{\text{gt}}) = \min \{ \text{BD}(L^{\text{ar}}, L^{\text{gt}}), \text{BD}(L^{\text{gt}}, L^{\text{ar}}) \}. \quad (3)$$

- Foreground–Background Dice (FBD), the Dice score of the foreground mask (i.e. the whole plant assuming the union of all leaf labels), to evaluate a delineation of a plant from the background obtained algorithmically with respect to the ground truth.

We should note that we also considered the Global Consistency Error (GCE) [32] and Object-level Consistency Error (OCE) [42] metrics, which are suited for evaluating segmentation of multiple objects. However, we found that they are harder to interpret and that the SBD is capable of capturing relevant leaf segmentation errors.

To evaluate how good an algorithm is in identifying the correct number of leaves present, we relied on:

- Difference in Count (DiC), the number of leaves in algorithm's result minus the ground truth:

$$\text{DiC} = \#L^{\text{ar}} - \#L^{\text{gt}}. \quad (4)$$

- $|\text{DiC}|$, the absolute value of DiC.

In addition to the metrics adopted for the challenge, in Sect. 7.5, we augment our evaluation here with metrics that prioritize leaf shape and boundary accuracy.

4 Leaf data and manual annotations

4.1 Imaging setup and protocols

We devised imaging apparatus, setups, and experiments emulating typical phenotyping experiments, to acquire three imaging datasets, summarized in Table 1, to support this study. They were acquired in two different labs with highly diverse equipment. Detailed information is available in [46], but brief descriptions are given below for completeness.

Arabidopsis data: Arabidopsis images were acquired in two data collections, in June 2012 and in September–October 2013, hereafter named *Ara2012* and *Ara2013*, respectively, both consisting of top-view time-lapse images of several *Arabidopsis thaliana* rosettes arranged in trays. Arabidopsis images were acquired using a setup for investigating affordable hardware for plant phenotyping.² Images were captured with a 7 megapixel consumer-grade camera (Canon PowerShot SD1000) during day time only, every 6 h over a period of 3 weeks for *Ara2012*, and every 20 min over a period of 7 weeks for *Ara2013*.

Acquired raw images (3108×2324 pixels, pixel resolution of ~ 0.167 mm) were first saved as uncompressed (TIFF) files, and subsequently encoded using the lossless compression standard available in the PNG file format [53].

Tobacco data: Tobacco images were acquired using a robotic imaging system for the investigation of automated plant treatment optimization by an artificial cognitive system.³ The robot head consisted of two stereo camera systems, black-and-white and colour, each consisting of 2 Point-Grey Grasshopper cameras with 5 megapixel (2448×2048 , pixel size $3.45 \mu\text{m}$) resolution and high-quality lenses (Schneider Kreuznach Xenoplan 1.4/17-0903). We added lightweight white and NIR LED light sources to the camera head.

Using this setup, each plant was imaged separately from different but fixed poses. In addition, for each pose, small baseline stereo image pairs were captured using each single camera, respectively, by a suitable robot movement, allowing for 3D reconstruction of the plant. For the top-view pose, distance between camera centre and top edge of pot varied between 15 and 20 cm for different plants, but being fixed

² <http://www.phenotiki.com>.

³ <http://www.garnics.eu/>.

Table 1 Summary of information of the Arabidopsis and tobacco experiments providing the three datasets

Experiment	Subjects	Wild-types	Mutants	Period (days)	Image resolution	Field of view	Per plant resolution
Ara2012	19	Col-0	No	21	7 MPixel	Whole tray	0.25 MPixel
Ara2013	24	Col-0	Yes (4)	49	7 MPixel	Whole tray	0.25 MPixel
Tobacco (23.01.2012)	20	Samsun	No	18	5 MPixel	Single plant	5 MPixel
Tobacco (16.02.2012)	20	Samsun	No	20	5 MPixel	Single plant	5 MPixel
Tobacco (15.05.2012)	20	Samsun	No	18	5 MPixel	Single plant	5 MPixel
Tobacco (10.08.2012)	20	Samsun	No	30	5 MPixel	Single plant	5 MPixel

per plant, resulting in lateral resolutions between 20 and 25 pixel/mm.

Data used for this study stemmed from experiments aiming at acquiring training data and contained a single plant imaged directly from above (top-view). Images were acquired every hour, 24/7, for up to 30 days.

4.2 Selection of data for training and testing

As part of the benchmark data for the LSC, we released three datasets, named respectively ‘A1’, ‘A2’, and ‘A3’, consisting of single-plant images of Arabidopsis and tobacco, each accompanied by manual annotation (discussed in the next section) of plant and leaf pixels. Examples are shown in Fig. 1.

From the Ara2012 dataset, to form the ‘A1’ dataset, we extracted cropped regions of 161 individual plants (500×530 pixels) from tray images, spanning a period of 12 days. An additional 40 images (530×565 pixels) form ‘A2’, which were extracted from the Ara2013 dataset spanning a period of 26 days. From the tobacco dataset, to form the ‘A3’ dataset, we extracted 83 images (2448×2048 pixels). Each dataset was split into training and testing sets for the challenge (cmp. Sect. 5).

The data differ in resolution, fidelity, and scene complexity, with plants appearing in isolation or together with other plants (in trays), with plants belonging to different cultivars (or mutants), and subjected to different treatments.

Due to the complexity of the scene and of the plant objects, the datasets present a variety of challenges with respect to analysis. Since our goal was to produce a wide variety of images, images corresponding to several challenging situations were included.

Specifically, in ‘A1’ and ‘A2’, on occasion, a layer of water from irrigation of the tray causes reflections. As the plants grow, leaves tend to overlap, resulting in severe leaf occlusions. Nastic movements (a movement of a leaf usually on the vertical axis) make leaves appear of different shapes and sizes from one time instant to another. In ‘A3’ beneath shape changes due to nastic movements, also different leaf shapes appear due to different treatments. Under

high illumination conditions (one of the treatment options in the Tobacco experiments), plants stay more compact with partly wrinkled, severely overlapping leaves. Under lower light conditions, leaves are more rounded and larger.

Furthermore, the ‘A1’ images of Arabidopsis present a complex and changing background, which could complicate plant segmentation. A portion of the scene is slightly out of focus (due to the large field of view) and appears blurred, and some images include external objects such as tape or other fiducial markers. In some images, certain pots have moss on the soil, or have dry soil and appear yellowish (due to increased ambient temperature for a few days). ‘A2’ presents a simpler scene (e.g. more uniform background, sharper focus, without moss); however, it includes mutants of Arabidopsis with different phenotypes related to rosette size (some genotypes produce very small plants) and leaf appearance with major differences in both shape and size.

The images of tobacco in ‘A3’ have considerably higher resolution, making computational complexity more relevant. Additionally, in ‘A3’, plants undergo a wide range of treatments changing their appearance dramatically, while Arabidopsis is known to have different leaf shape among mutants. Finally, other factors such as self-occlusion, shadows, leaf hairs, and leaf colour variation make the scene even more complex.

4.3 Annotation strategy

Figure 2 depicts the procedure we followed to annotate the image data. In the first place, we obtained a binary segmentation of the plant objects in the scene in a computer-aided fashion. For Arabidopsis, we used the approach based on

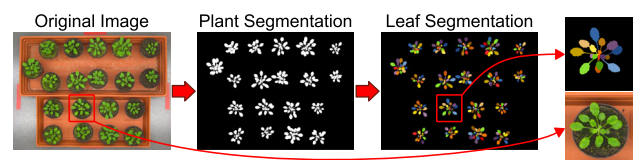


Fig. 2 Schematic of the workflow to annotate the images. Plants were first delineated in the original image and then individual leaves were labelled

active contours described in [34], while for tobacco, a simple colour-based approach for plant segmentation was used. The result of this segmentation was manually refined using raster graphics editing software. Next, within the binary mask of each plant, we delineated individual leaves, following an approach completely based on manual annotation. A pixel with black colour denotes background, while all other colours are used to uniquely identify the leaves of the plants in the scene. Across the frames of the time-lapse sequence, we consistently used the same colour code to label the occurrences of the same leaf. The labelling procedure involved always two annotators to reduce observer variability, one annotating the dataset and one inspecting the other.

Note that LSC did not involve leaf tracking over time, therefore all individual plant images were considered separately, ignoring any temporal correspondence.

4.4 File types and naming conventions

Plant images were encoded using the lossless PNG [53] format and their dimensions varied. Plant objects appeared centred in the (cropped) images. Segmentation masks were image files encoded as indexed PNG, where each segmented leaf was identified with a unique (per image) integer value, starting from '1', whereas '0' denotes background. The union of all pixel labels greater than zero provides the ground truth plant segmentation mask. A colour index palette was included within the file for visualization reasons. The file-names have the form:

- plantXXX_rgb: the original RGB colour image;
- plantXXX_label: the labelled image;

where XXX is an integer number. Note that plants were not numbered sequentially.

5 CVPPP 2014 LSC challenge outline

The LSC challenge was organized by two of the authors (HS and SAT), as part of the CVPPP workshop, which was held in conjunction with the European Conference on Computer Vision (ECCV), in Zürich, Switzerland, in September 2014. Electronic invitations for participation were communicated to a large number of researchers working on computer vision solutions for plant phenotyping and via computer vision and pattern recognition mailing lists and several phenotyping consortia and networks such as DPPN,⁴ IPPN,⁵ EPPN,⁶

iPlant.⁷ Interested parties were asked to visit the website and register for the challenge after agreeing to rules of participation and providing contact info via an online form.

Overall, 25 teams registered for the study and downloaded training data, 7 downloaded testing data, and finally 4 submitted manuscript and testing results for review at the workshop. For this study, we invited several of the participants (see Sect. 6).

5.1 Training phase

An example preview of the training set (i.e. one example image from each of the three datasets as shown in Fig. 1) was released in March 2014 on the CVPPP 2014 website. The full training set, consisting of colour images of plants and annotations, was released in April 2014.

A total of 372 PNG images were available in 186 pairs of raw RGB colour images and corresponding annotations in the form of indexed images, namely 128, 31, and 27 images for 'A1', 'A2', and 'A3', respectively. Images of many different plants were included at different time points (growth stages). Participants were unaware of any temporal relationships among images, and were expected to treat each image in an individual fashion. Participants were allowed to tailor pipelines to each dataset and could choose supervised or unsupervised methods. Matlab evaluation functions were also provided to help participants assess performance on the training set using the criteria discussed in Sect. 3. The data and evaluation script are in the public domain.⁸

5.2 Testing phase

We released 98 colour images for testing (i.e. 33, 9, and 56 images from 'A1', 'A2', and 'A3', respectively) and kept the respective label images hidden. Images here corresponded to plants at different growing stages (with respect to those included in the training set) or completely new and unseen plants. Again this was unknown to the participants. A short testing period was allowed: the testing set was released on June 9, 2014, and authors were asked to submit their results by June 17, 2014, and accompanying papers by June 22, 2014.

In order to assess the performance of their algorithm on the test set, participants were asked to email to the organizers a ZIP archive following a predefined folder/file structure that enabled automated processing of the results. Within 24 h, all participants who submitted testing results received their evaluation using the same evaluation criteria as for training, as well as summary tables in L^AT_EX and also individual per-image results in a CSV format. Algorithms and the papers

⁴ <http://www.dppn.de/>.

⁵ <http://www.plant-phenotyping.org/>.

⁶ <http://www.plant-phenotyping-network.eu/>.

⁷ <http://www.iplantcollaborative.org/>.

⁸ <http://www.plant-phenotyping.org/CVPPP2014-dataset>.

were subject to peer review and the leading algorithm [41] presented at the CVPPP workshop.

6 Methods

We briefly present the leaf segmentation methods used in this collation study. We include methods not only from challenge participants but also others for completeness and for offering a larger view of the state of the art. Overall, three methods rely on post-processing of distance maps to segment leaves, while one uses a database of templates which are matched using a distance metric. Each method's description aims to provide an understanding of the algorithms, and wherever appropriate, we offer relevant citations for readers seeking additional information.

Please note that participating methods were given access to the training set (including ground truth) and testing set but without ground truth.

6.1 IPK Gatersleben: segmentation via 3D histograms

The IPK pipeline relies on unsupervised clustering and distance maps to segment leaves. Details can be found in [41]. The overall workflow is depicted in Fig. 3 and summarized in the following.

1. Supervised foreground/background segmentation utilizing 3D histogram cubes, which encode the probability for any observed pixel colour in the given training set of belonging to the foreground or background; and
2. Unsupervised feature extraction of leaf centre points and leaf split point detection for individual leaf segmentation by using a distance map, skeleton, and the corresponding graph representation (cmp. Fig. 3).

To avoid the partitioning of the 3D histogram in rectangular regions [30], here a direct look-up in the 3D histogram cubes instead of (multiple) one-dimensional colour component thresholds is used. For this approach, two 3D histogram cubes for foreground and background are accumulated using the provided training data. To improve the performance against illumination variability, input images are converted into the *Lab* colour space [7]. Entries which are not represented in the training data are estimated by using an interpolation of the surrounding values of a histogram cell. The segmentation results are further processed by morphological operations and cluster-analysis to suppress noise and artefacts. The outcome of this operation serves as input for the feature extraction phase to detect leaf centre points and optimal split points of corresponding leaf segments.

For this approach, the leaves of *Arabidopsis* plants in 'A1' and 'A2' are considered as compact objects which only partly overlap. In the corresponding Euclidean distance map

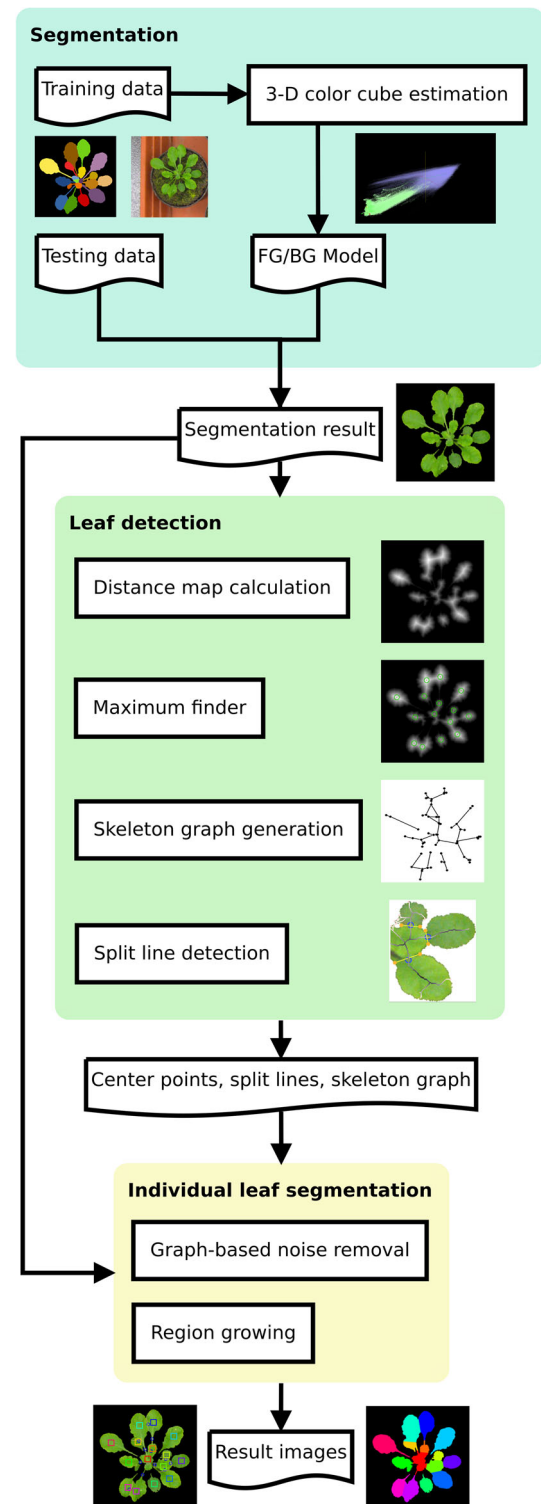


Fig. 3 Workflow of the IPK approach, including main processing components: segmentation, image feature extraction (including leaf detection), and individual leaf segmentation

(EDM), the leaf centre points appear as peaks, which are detected by a maximum search. At the next step, a skeleton image is calculated. To resolve leaf overlaps, split points

at the thinnest connection points are detected. Values of the EDM are mapped on the skeleton image. The resulting image is used for creating a skeleton graph, where leaf centre points, skeleton end-points, and skeleton branch-points are represented as nodes in the graph. Edges are created if the corresponding image points are connected by a skeleton line. Additionally, a list of the positions and minimal distances of each particular edge segment are saved as an edge-attribute. This list is used to detect the exact positions of the leaf split points. In order to find the split point(s) between two leaf centre points, all nodes and edges of the graph structure connecting these two points are traversed and the position with the minimal EDM values is determined. This process is repeated, if there are still connections between the two leaves which need to be separated. For calculating the split line belonging to a particular minimal EDM point, two coordinates on the plant leaf border are calculated. The nearest background pixel is searched (first point), and also the nearest background pixel at the opposite position relative to the split point (second point) is located. The connection line is used as border during the segmentation of overlapping leaves. In a final step, the separated leaves are labelled by a region growing algorithm.

Our approach was implemented in Java, and tested on a desktop PC with 3.4 GHz processor and 32 GB memory. Java was configured to use a maximum of 4 GB RAM. On average each image takes 1.6, 1.2, and 9 seconds for ‘A1’, ‘A2’, and ‘A3’, respectively.

6.2 Nottingham: segmentation with SLIC superpixels

A superpixel-based method that does not require any training is used. The training dataset has been used for parameter tuning only. The processing steps visualized in Fig. 5 can be summarized as follows:

1. Superpixel over-segmentation in *Lab* colour space using SLIC [1];
2. Foreground (plant) extraction using simple seeded region growing in the superpixel space;
3. Distance map calculation on extracted foreground;
4. Individual leaf seed matching by identifying the superpixels whose centroid lays in the local maxima of the distance map; and
5. Individual leaf segmentation by applying watershed transform with the extracted seeds.

Steps (1) and (2) are used to extract the whole plant while (3), (4) and (5) for extracting the individual leaves. We now present a detailed explanation of each of the steps, with Figs. 4 and 5 summarizing the process.

Preparation. Given an RGB image, it is first converted to the *Lab* colour space [7], to enhance discrimination between

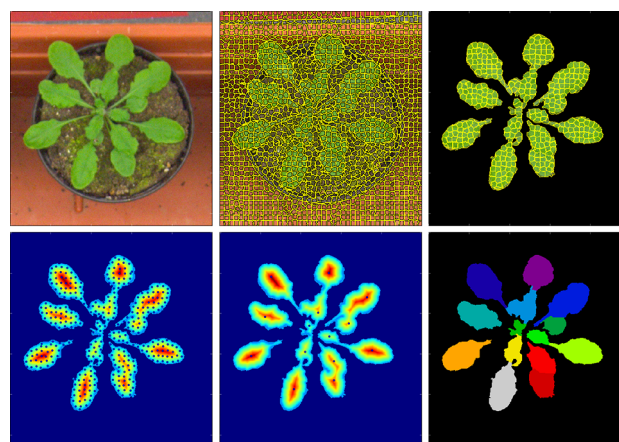


Fig. 4 Example images of each of the steps in the Nottingham approach. *First row* original image (*left*), SLIC superpixels (*centre*), thresholded superpixels (*right*). *Second row* distance map with superpixel centroids (*left*), filtered superpixel centroids (*middle*), watershed segmentation (*right*)

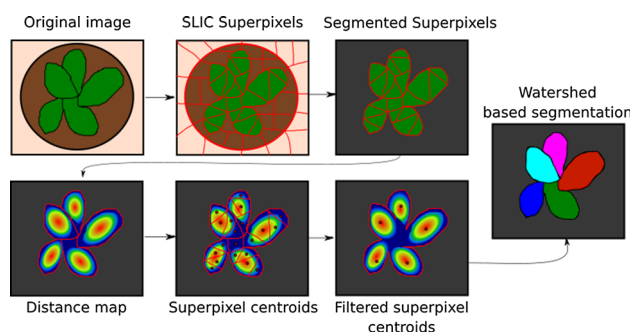


Fig. 5 The Nottingham leaf segmentation process

foreground leaves and background. Then, SLIC superpixels [1] are computed. A fixed number of superpixels are computed over the image. Empirically, 2000 pixels was found to provide good coverage of the leaves. The mean colour of the ‘a’ channel (which characterizes the green colour of the image well) is extracted for each superpixel. A Region Adjacency Map (superpixel neighbourhood graph) is created from the resulting superpixels.

Foreground extraction. Having the mean colour of each superpixel for channel ‘a’, a simple region growing approach [2] in superpixel space allows the complete plant to be extracted. The superpixel with the lowest mean colour (the most bright green superpixel) defined in *Lab* space is used as the initial seed. However, for ‘A1’ and ‘A2’, since they do not contain shadows, an even simpler thresholding of the mean colour of each superpixel allows faster yet still accurate segmentation of the plant. Thresholds for the ‘A1’ and ‘A2’ are set to -25 and -15 respectively.

Leaf identification. Once the plant is extracted from the background, superpixels not belonging to the plant are removed. A distance map is computed (first removing strong

edges using the Canny detector [11]) and the centroids are calculated for all superpixels. A local maxima filter is applied to extract the superpixels that lay in the centre of the leaves. A superpixel is selected as a seed only if it is the most central in the leaf compared to its neighbours within a radius. This is implemented by considering the superpixel centroid value in the distance map, and filtering the superpixels that do not have the maximum value within its neighbours.

Leaf segmentation. Finally, watershed segmentation [51] is applied with the obtained initial seeds over the image space, yielding the individual leaf segmentation.

Using a Python implementation running on a i3 quad-core desktop with i3-4130 (3.4 GHz) processor and 8 GB memory, on average, each image takes < 1 second for dataset ‘A1’ and ‘A2’, and 1–5 seconds for ‘A3’.

Overall, it is a fast method with no training required. It also could be tuned to get a much higher accuracy on a per-image basis. The parameters that can be tuned are as follows: (1) number of superpixels, (2) compactness of superpixels, (3) foreground extractor (threshold or region growing), (4) parameters of the canny edge detector, and (5) colour space for SLIC, foreground extractor and canny edge detector. All those parameters were tuned in a per-dataset basis using the training set in order to maximize the mean Symmetric Best Dice score for each dataset. However, they can be easily tuned manually on a per-image basis if required.

6.3 MSU: leaf segmentation with Chamfer matching

The MSU approach extends a multi-leaf alignment and tracking framework [59–61] to the LSC. As discussed in Sect. 2, this framework was originally designed for segmenting and tracking leaves in plant fluorescence videos where plant segmentation is straightforward due to the clean background. For the LSC, a more advanced background segmentation process was adopted.

The framework is motivated by the well-known Chamfer Matching (CM) [8], which aligns one object instance in an image with a given template. However, since there are large variations of leaves in plant images, it is infeasible to match leaves with only one template. Therefore, we generate a set of templates with different shapes, scales, and orientations. Specifically, H leaves with representative shapes (e.g. different aspect ratios) are selected from H images of the training set. Each leaf shape is scaled to S different sizes, and each size is rotated to R different orientations. This leads to a set of $H \times S \times R$ leaf templates ($5 \times 9 \times 24$ for ‘A1’ and ‘A2’, $8 \times 10 \times 24$ for ‘A3’) with labelled tip locations, which will be used in the segmentation process.

An accurate plant segmentation and edge map are critical to obtain reliable CM results. To this end, all RGB images are converted into the *Lab* colour space, and a threshold τ is applied to channel ‘a’ for estimating a foreground mask

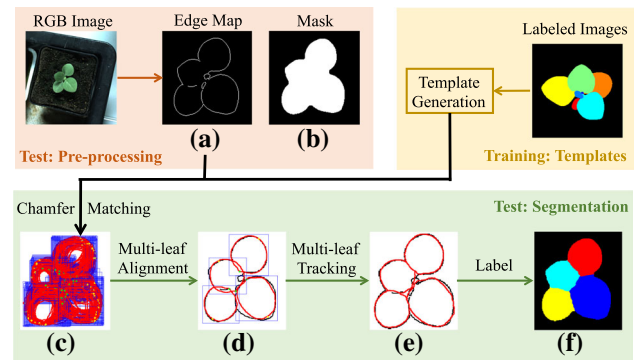


Fig. 6 Overview of the MSU approach: training is done once for each plant type (i.e. twice for three datasets), and pre-processing and segmentation are performed for each image

(chosen empirically for each dataset: 40 for ‘A1’, and 30 for ‘A2’ and ‘A3’), which is refined by standard morphological operations. The Sobel edge operator is applied within the foreground segment to generate an edge map. Since ‘A3’ has more overlapping leaves and the boundaries between the leaves are more visible due to shadows, an additional edge map is used, obtained by applying the edge operator on the image resulting from the difference of ‘a’ and ‘b’ channels.

Morphological operations are applied to remove small edges (noise) and lines (leaf veins). A mask (Fig. 6a) and edge map (Fig. 6b) are cropped from the RGB image.

For each template, we search all possible locations on the edge map and find one location with the minimum CM distance. Doing so for all templates generates an overcomplete set of leaf candidates (Fig. 6c). For each leaf candidate, we compute the CM score, its overlap with foreground mask, and the angle difference, which measures how well the leaf points to the centre of the plant. Our goal is to select a subset of leaf candidates as the segmentation result. First, we delete candidates with large CM scores, small overlap with the foreground mask, or a large angle difference. Second, we develop an optimization process [61] to select an optimal set of leaf candidates by optimizing the minimal number of candidates with smaller CM distances and leaf angle differences to cover the foreground mask as much as possible. Third, all leaf candidates are selected as an initialization and gradient descent is applied to iteratively delete redundant leaf candidates, which leads to a small set of final leaf candidates.

As shown in Fig. 6d, a finite number of templates cannot perfectly match all edges. We apply a multi-leaf tracking procedure [60] to transform each template, i.e. rotation, scaling, and translation, to obtain an optimal match with the edge map. This is done by minimizing the summation of three terms: the average CM score, the difference between the synthesized mask of all candidates and the test image mask, and the average angle difference. The leaf alignment result provides initialization of the transformation parameters and gradient

descent is used to update these parameters. When a leaf candidate becomes smaller than a threshold, we will remove it. After this optimization, the leaf candidates will match the edge map much more accurately (Fig. 6e), which remedies the limitation of a finite set of leaf templates. Finally, we use the tracking result and foreground mask to generate a label image so that all foreground pixels, and only foreground pixels, have labels.

Only one leaf out of each of the H training images is used for template generation. The same pre-processing and segmentation procedures are conducted independently for each image of the training and testing set.

Using our Matlab implementation running on a quad-core desktop with 3.40GHz processor and 32GB memory, on average each image takes 63, 49, and 472 seconds for ‘A1’, ‘A2’, and ‘A3’ respectively.

6.4 Wageningen: leaf segmentation with watersheds

The method consists of two steps: plant segmentation and separate leaf segmentation, illustrated in Fig. 7. Plant segmentation from the background uses supervised classification with a neural network. Since the nature of the three datasets (‘A1’, ‘A2’, and ‘A3’) is different, a separate classifier and post-processing steps are applied to each individual set. The ground truth images are used to mask plant and background pixels. For all images, 3000 pixels of each class are randomly selected for training. When the plant is smaller than 3000 pixels, all plant pixels are used. To separate the plants from the background, four colour and two texture features are used for each pixel. The colour features used in the classification are red, green, and blue pixel values (R, G, B) and

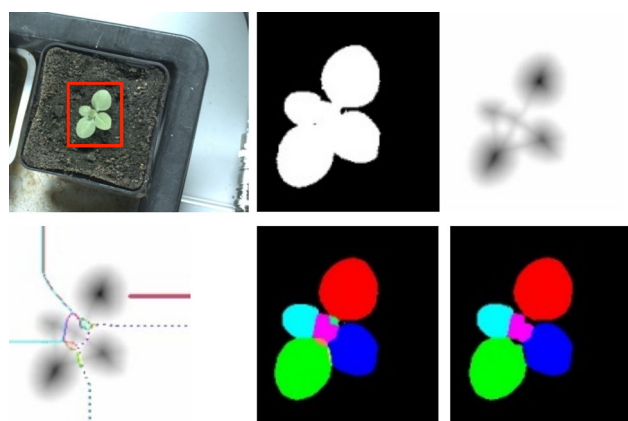


Fig. 7 Steps of the Wageningen approach, shown on an image from ‘A3’ (top left, with zoomed detailed shown in red box): test RGB image (top left), neural network-based foreground segmentation (top middle), inverse distance image transform (top right), watershed basins (bottom left), intersection of basins and the foreground image mask (bottom middle), final leaf segmentation after rejecting small regions (bottom right) (colour figure online)

the excessive Green value ($2G-R-B$) which highlights green pixels. For texture features, the pixel values of the variance filtered green channel [62], and the pixel values of the gradient magnitude filtered green channel are used. The latter two highlight edges and rough parts in the image.

A large range of linear and nonlinear classifiers have been tested on each dataset, with a feed-forward (MLP) neural network with one hidden layer of 10U giving the best results. Morphological operations are applied on the binary image obtained after plant classification, resulting in the plant masks (i.e. a foreground–background segmentation). For ‘A1’ and ‘A2’, the morphological operations consist of an erosion followed by a propagation using the original results as mask. Small blobs mainly from moss are removed this way. For ‘A3’, all blobs in the image are removed, except for the largest one.

In order to remove moss that occurs in ‘A2’ and ‘A3’ and in order to emphasize spaces between stems and leaves (cf. Fig. 8) to which the watershed algorithm is highly sensitive, additional colour transformation, shape and spatial filtering, and morphological operations are applied. For ‘A2’, all components of the foreground segmentation are filtered out that are further away from the centre of gravity of the foreground mask than 1.5 times estimated radius of the foreground mask. The radius r is estimated from mask area A as $r = (A/\pi)^{\frac{1}{2}}$. Next, the Y-component image of the YUV colour transformation, giving the luminance, is thresholded with a threshold optimized on the training set ($th = 85$). For ‘A3’, there are cases of large moss areas attached to the foreground segmentation mask. To remove them, first the compactness C of the foreground mask is calculated as $C = L^2/(4\pi A)$, where L is the foreground mask contour length. $C > 20$ indicates the presence of a large moss area segmented as foreground. There, the X-component of the XYZ colour transformation yielding chromatic information is thresholded ($th = 55$), and the pixels that are smaller than the threshold are filtered out. In this way, the moss pixels which have a slightly different colour than the plants are removed from the foreground image. Next, in order to emphasize spaces between the leaves and the stems, all foreground masks are corrected with the

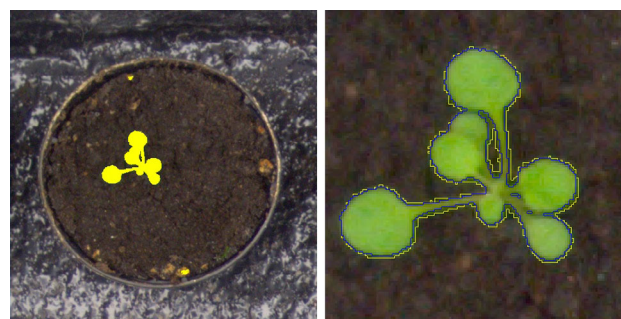


Fig. 8 Wageningen: accentuating holes

thresholded Y-component of the YUV colour-transformed image as described for ‘A2’.

The second step, i.e. separate leaf segmentation, is performed using a watershed method [9] applied on the Euclidean distance map of the resulting plant mask image from the first step of the method. Initially, the watershed transformation is computed without applying the threshold between the basins. In the second step, the basins are successively merged if they are separated by a watershed that is smaller than a given threshold. The threshold value is tuned on the training set in order to produce the best result. The thresholds are set to 30, 58, and 70 for the datasets ‘A1’, ‘A2’, and ‘A3’ respectively.

Plant segmentation is done in Matlab 2015a and the perClass classification toolbox (<http://perclass.com>) on a MacBook with 2.53 GHz Intel Core 2 Duo. Learning the neural network classifier using a training set of 6000 pixels takes about 4 s per image. Plant segmentation using this trained classifier and post-processing take 0.76 s, 0.73 s and 24 s for ‘A1’, ‘A2’, and ‘A3’ respectively. Moss removal and leaf segmentation are performed in Halcon, running on a laptop with 2.70 GHz processor and 8 GB memory. On average, each image takes 160, 110, and 700 ms for ‘A1’, ‘A2’, and ‘A3’ respectively.

7 Results

In this section, we discuss the performance of each method as evaluated on testing and training sets. Note that the ground truth was available to participants (authors of this study) for the training set; however, the testing set was only known to the organizers of the LSC (i.e. S. A. Tsafaris, H. Scharr, and M. Minervini) and was unavailable to all others. Training set numbers are provided by the participants (with the same evaluation function and metrics used also on the testing set).

Note that since Nottingham is an unsupervised method, the results reflect directly the performance on all the training set. With MSU, since they use some of the leaves in the training set to define their templates some bias could exist, but it is minimal. IPK and Wageningen apply supervised methods to obtain foreground segmentation, using the whole dataset (Wageningen use a random selection of 3000 pixels per class per image).

7.1 Plant segmentation from background

Figure 9 shows selected examples of test images from the three datasets. We choose from each dataset two examples: one to show the effectiveness of the methods and one to show limitations. We show visually the segmentation outcomes for each method together with ground truth; we also overlay the numbers of the evaluation measures on the images.

Overall, we see that most methods perform well in separating plant from background, except when the background presents challenges (e.g. moss) as does the second image shown for ‘A1’. Then FBD scores are lower for almost all methods, with IPK and Nottingham showing more robustness. These observations are evident in the whole dataset (cf. FBD numbers in Tables 2, 3). Average testing numbers are lower than training for most methods with the exception of Nottingham, which does significantly better in ‘A2’ and ‘A3’ in the testing case. Given that their method is unsupervised, this behaviour is not unexpected.

7.2 Leaf segmentation and counting

Referring again to Fig. 9 and Tables 2, 3, let us evaluate visually and quantitatively how well algorithms do in segmenting leaves. When leaves are not overlapping, all methods perform well. Nevertheless, each method exhibits different behaviour. IPK, MSU, and Wageningen obtain higher SBD scores; however, IPK does produce straight line boundaries that are not natural—they should be more curved to better match leaf shape. There seems to be also an interesting relationship between segmentation error and leaf size (see also next section for effects related to plant size).

In fact, plotting leaf size vs. Dice per leaf,⁹ see Fig. 10, we observe that with all methods larger leaves are more accurately delineated, with exception of the largest few leaves in MSU. Dice for smaller leaves shows more scatter and smaller leaves are more frequently not detected, as evidenced by the high symbol density at Dice = 0 (dark blue symbols). For small leaves with $(\text{leaf area})^{\frac{1}{2}} \lesssim 20$ Wageningen performs best, detecting more leaves than the others and with higher accuracy. IPK shows better performance than others in the mid range $40 \lesssim (\text{leaf area})^{\frac{1}{2}} \lesssim 80$ due to higher per-leaf accuracy (see the more dark/black symbols in the region above Dice = 0.95) and fewer non-detected leaves. In the mid range, only Wageningen performs similarly with respect to leaf detection (fewest symbols at Dice = 0), closely followed by MSU.

We should note that measuring SBD and FBD with Dice does have some limitations. If a method reports a Dice score of 0.9, this loss of 0.1 can be attributed to either an under-segmentation (e.g. loss of a stem in Arabidopsis, non-precise leaf boundary) or an over-segmentation (considering background as plant). Therefore, in Sect. 7.5, we apply two measures being more sensitive to shape consistency, in order to investigate the solutions’ performance with respect to leaf boundaries.

⁹ To measure Dice per leaf, we first find matches between a leaf in ground truth and an algorithm’s result that maximally overlap, and then report the Dice (Eq. 1) of matched leaves; for non-matched leaves a zero is reported.

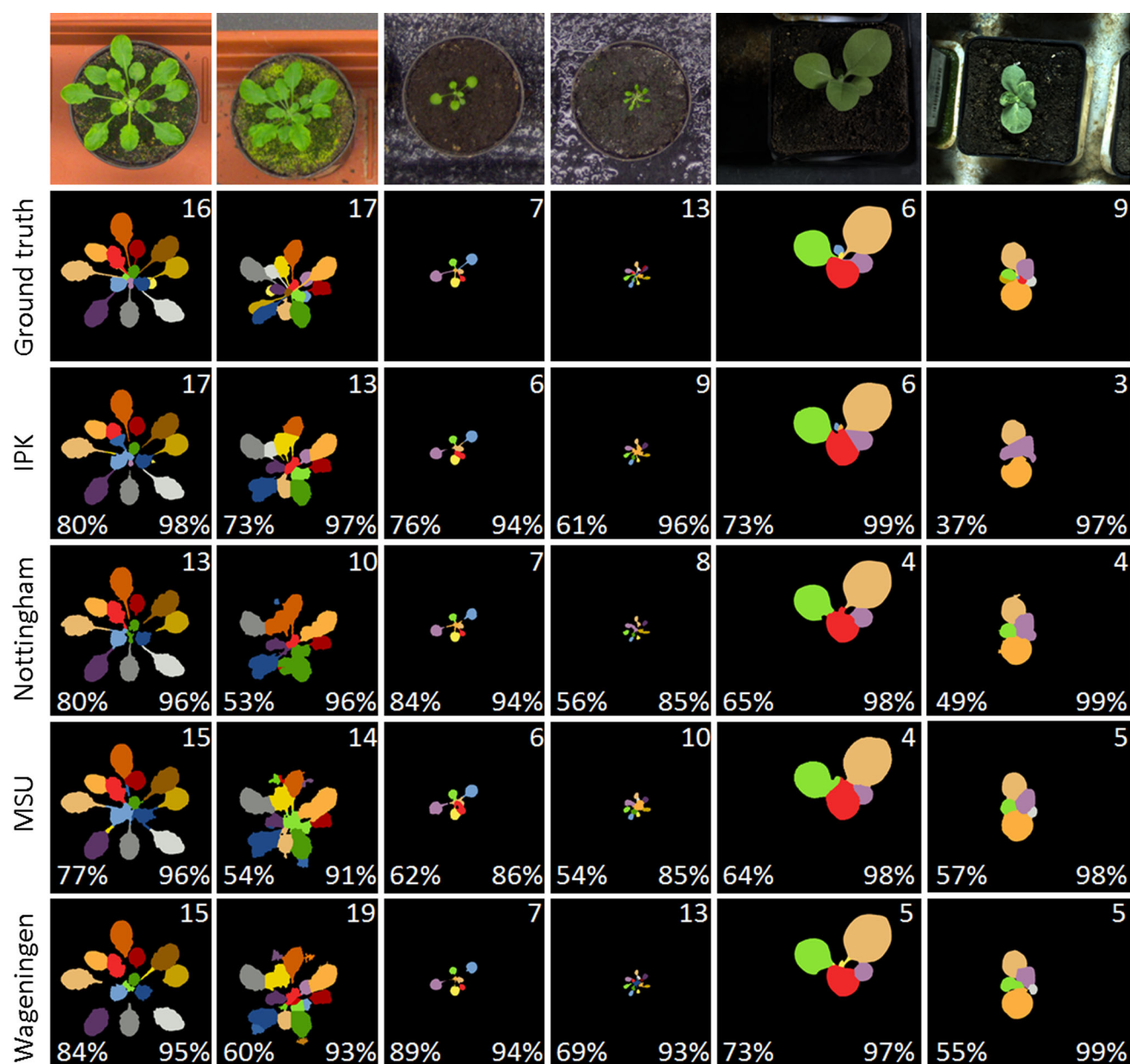


Fig. 9 Selected results on test images. From each dataset ‘A1’–‘A3’, an easier and more challenging image is shown, together with ground truth, and results of IPK, Nottingham, MSU, and Wageningen (from top to bottom, respectively). Numbers in the image corners are num-

ber of leaves (*upper right*), SBD (*lower left*), and FBD (*lower right*). For viewing ease, matching leaves are assigned the same colour as the ground truth. Figure best viewed in colour (colour figure online)

With regard to leaf counting, most methods show their limitations, and in fact using such a metric also highlights errors in leaf segmentation. For example, in Fig. 9, we see that when the images are more challenging, some methods merge leaves: this lowers SBD scores but affects count numbers even more critically. Other methods (e.g. Wageningen) tend to over-segment and consider other parts as leaves (see for example the second image of ‘A1’ in Fig. 9), which sometimes leads to over estimating numbers.

These misestimations are evident throughout training and testing sets (cf. Tables 2, 3). Stepping away from the summary statistics of the tables, over and under estimation are readily apparent in Fig. 11. All algorithms present counting outliers, where MSU yields the least count variability, despite a clear underestimation. The mean DiC of Wageningen is the closest to zero, albeit featuring the highest variances. We also observe that DiC lowers as the number of leaves increases, particularly in the case of ‘A3’.

Table 2 Segmentation and counting results on the training set

	SBD (%)	FBD (%)	DiC	DiC
IPK				
A1	74.2 (7.7)	97.4 (1.8)	2.6 (1.8)	−1.9 (2.5)
A2	80.6 (8.7)	99.7 (0.3)	0.9 (1.0)	−0.3 (1.3)
A3	61.8 (19.1)	98.2 (1.1)	2.1 (1.7)	−2.1 (1.7)
ALL	73.5 (11.5)	98.0 (1.9)	2.2 (1.7)	−1.7 (2.3)
Nottingham				
A1	68.0 (7.4)	94.6 (1.6)	3.8 (2.0)	−3.6 (2.4)
A2	60.9 (18.5)	87.5 (19.7)	2.5 (1.5)	−2.5 (1.5)
A3	47.1 (25.0)	79.4 (34.5)	2.3 (1.8)	−2.3 (1.9)
ALL	63.8 (15.3)	91.2 (16.2)	3.4 (2.0)	−3.2 (2.2)
MSU				
A1	78.0 (6.4)	95.8 (1.9)	2.3 (1.5)	−2.3 (1.6)
A2	72.3 (9.5)	94.1 (4.1)	1.6 (1.4)	−1.3 (1.7)
A3	69.6 (16.5)	95.0 (6.5)	1.4 (1.5)	−1.3 (1.5)
ALL	75.8 (9.6)	95.4 (3.4)	2.1 (1.5)	−2.0 (1.7)
Wageningen				
A1	72.8 (7.8)	95.0 (2.4)	2.2 (2.0)	0.4 (3.0)
A2	71.7 (8.0)	95.2 (2.4)	1.3 (1.1)	−0.6 (1.6)
A3	69.6 (19.9)	96.1 (5.1)	1.7 (2.4)	0.6 (2.9)
ALL	72.2 (10.5)	95.2 (3.0)	2.0 (2.0)	0.3 (2.8)

Average values are shown for metrics described in Sect. 3 and in parenthesis standard deviation. ‘ALL’ denotes the average (and standard deviation) among the three datasets for each method. Other shorthands and abbreviations as defined in text (Sects. 3, 6)

The best results for each metric are denoted in bold

7.3 Plant growth and complexity

Plants are complex and dynamic organisms that grow over time, and move throughout the day and night. They grow differentially, with younger leaves growing faster than mature ones. Therefore, per-leaf growth is a better phenotyping trait when evaluating growth regulation and stress situations. As they grow, new leaves appear and plant complexity changes: in tobacco, more leaves overlap and exhibit higher nastic movements; and in Arabidopsis, younger leaves emerge, overlapping other more mature ones.

At an individual leaf level, the findings of Fig. 10—Dice of smaller leaves showing higher variability scatter and with smaller leaves being missed—illustrate that we need to achieve homogeneous performance and robustness if we want to obtain accurate per-leaf growth estimates.

Using classical growth stages, which rely on leaf count as a marker of growth, the downwards slope seen in Fig. 11 could be attributed to growth. This is more clear in Fig. 12, where we see that with more leaves, leaf segmentation accuracy (SBD) also decreases.

Even if we consider plant size (measured as PLA, i.e. the size of the plant in ground truth, obtained as the union of all

Table 3 Segmentation and counting results on the testing set

	SBD (%)	FBD (%)	DiC	DiC
IPK				
A1	74.4 (4.3)	97.0 (0.8)	2.2 (1.3)	−1.8 (1.8)
A2	76.9 (7.6)	96.3 (1.7)	1.2 (1.3)	−1.0 (1.5)
A3	53.3 (20.2)	94.1 (13.3)	2.8 (2.5)	−2.0 (3.2)
ALL	62.6 (19.0)	95.3 (10.1)	2.4 (2.1)	−1.9 (2.7)
Nottingham				
A1	68.3 (6.3)	95.3 (1.1)	3.8 (1.9)	−3.5 (2.4)
A2	71.3 (9.6)	93.0 (4.2)	1.9 (1.7)	−1.9 (1.7)
A3	51.6 (16.2)	90.7 (20.4)	2.5 (2.4)	−1.9 (2.9)
ALL	59.0 (15.6)	92.5 (15.6)	2.9 (2.3)	−2.4 (2.8)
MSU				
A1	66.7 (7.6)	94.0 (1.9)	2.5 (1.5)	−2.5 (1.5)
A2	66.6 (7.9)	87.7 (3.6)	2.0 (1.5)	−2.0 (1.5)
A3	59.2 (17.8)	95.0 (5.2)	2.3 (1.9)	−2.3 (1.9)
ALL	62.4 (14.8)	94.0 (4.7)	2.4 (1.7)	−2.3 (1.8)
Wageningen				
A1	71.1 (6.2)	94.7 (1.5)	2.2 (1.6)	1.3 (2.4)
A2	75.7 (8.4)	95.1 (2.0)	0.4 (0.5)	−0.2 (0.7)
A3	57.6 (24.8)	89.5 (22.3)	3.0 (4.9)	1.8 (5.5)
ALL	63.8 (20.5)	91.7 (17.0)	2.5 (3.9)	1.5 (4.4)

Shorthands and abbreviations as in Table 2

The best results for each metric are denoted in bold

leaf masks), we observe a decreasing trend in SBD for each method with plant size, see Fig. 13. Observe the large variability in SBD when plants are smaller. Even isolating it to a single method we see that when plants are small, depending on the plant’s leaf arrangement, variability is extremely high: either good (close to 80 %) or rather low SBD values are obtained.

7.4 Effect of foreground segmentation accuracy

In Fig. 14, we plot FBD versus SBD for each method pooling the testing data together. We see that high SBD can only be achieved when FBD is also high; but obtaining a high FBD is not at all a guarantee for good leaf segmentation (i.e. a high SBD) since we observe large variability in SBD even when FBD is high.

This prompted us to evaluate the performance of the leaf segmentation part isolating it from errors in the plant (foreground) segmentation step. Thus, we asked participants to submit results on the training set assuming that also a foreground (plant) mask is given (as obtained by the union of leaf masks), effectively not requiring a plant segmentation step.

Naturally, all methods benefit when the ground truth plant segmentation is used: compare SBD, DiC, and |DiC| between Tables 2 and 4. SBD improves considerably in most

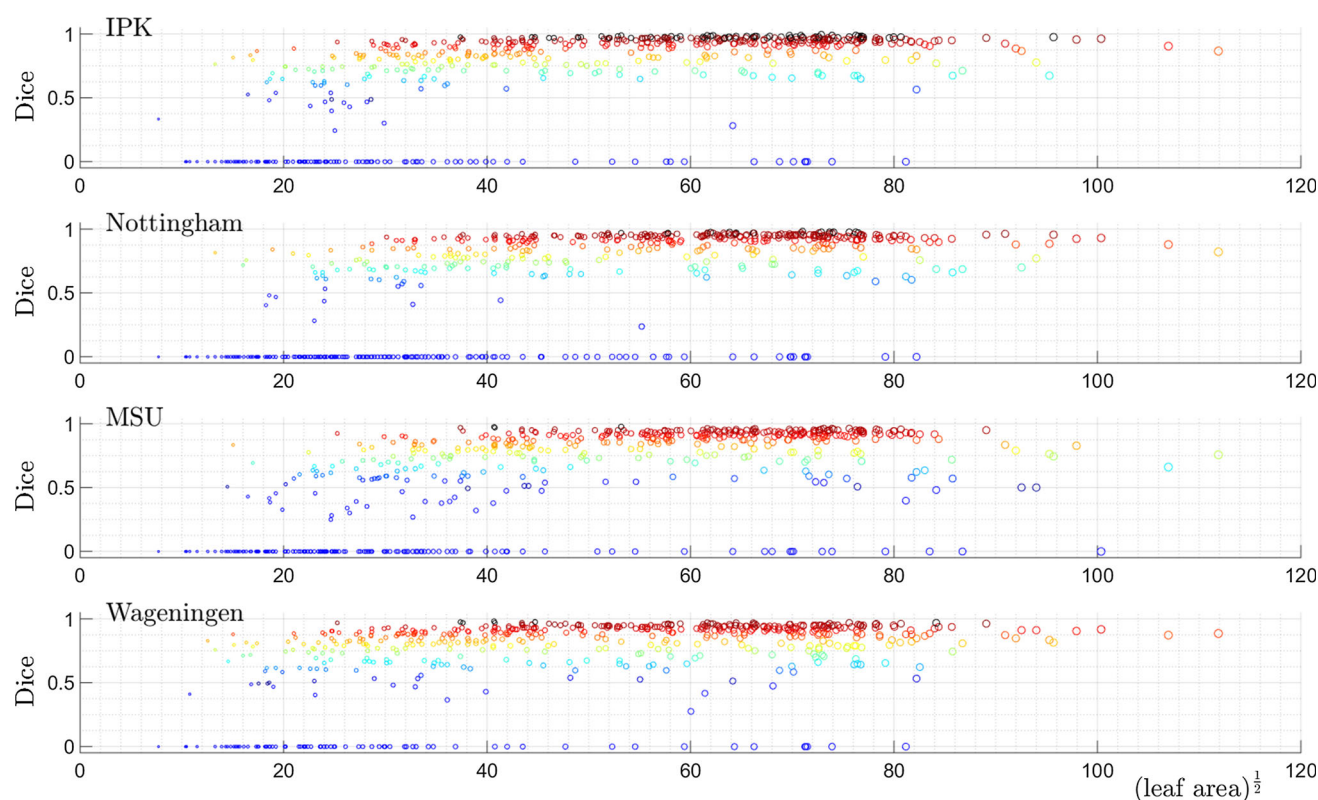


Fig. 10 Dataset ‘A1’: Dice score per leaf versus $(\text{leaf area})^{1/2}$, i.e. ground truth average leaf radius (in pixels). Larger symbols refer to larger leaves. Colour also indicates Dice score for better visibility. Figure best viewed in colour (colour figure online)

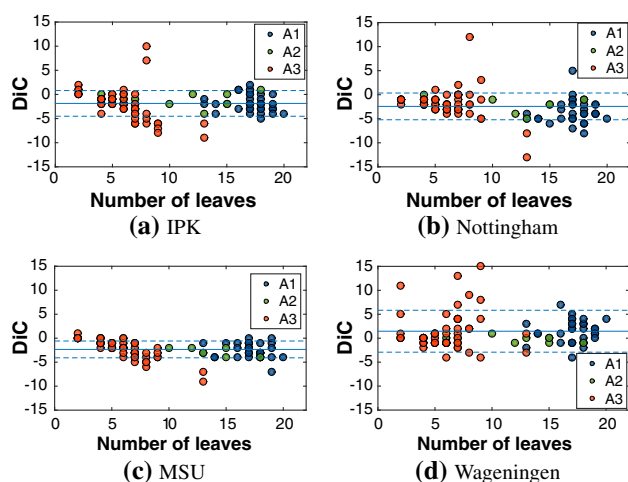


Fig. 11 For each method, scatters of number of leaves in ground truth versus Difference in Count (DiC) are shown for the testing set. Each dataset is colour coded differently. Also, lines of average and average \pm one standard deviation of DiC are shown, as solid and dashed blue lines, respectively (colour figure online)

cases; counting improvement is less pronounced, and sometimes results even get worse. Best performance measures are achieved by IPK (closely followed by MSU) for SBD and Wageningen for counting. Note that for ‘A3’ IPK’s, SBD performance increases substantially with known plant seg-

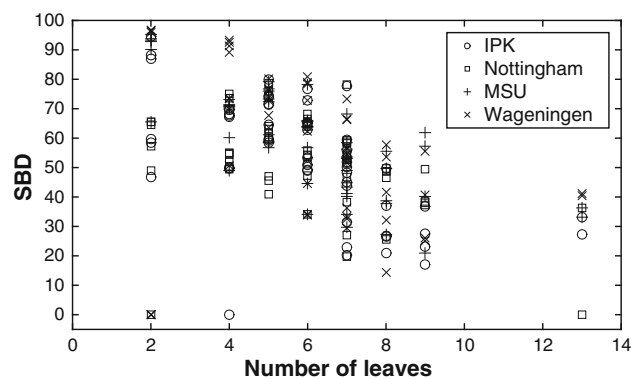


Fig. 12 Effect of plant complexity (measured as number of leaves) on leaf segmentation accuracy, i.e. SBD, for ‘A3’. Each method is marker coded separately

mentation. Overall, additional investment in obtaining better performing foreground segmentation is therefore warranted.

Comparing the count numbers (DiC and |DiC| in Tables 2, 4), the best performer is Wageningen, with slight over-estimation in Table 2 and slight under-estimation Table 4, while again all other methods under-estimate the number of leaves present. Even when foreground plant mask is given, these numbers do not improve significantly. So it is not errors in the foreground segmentation component that cause such

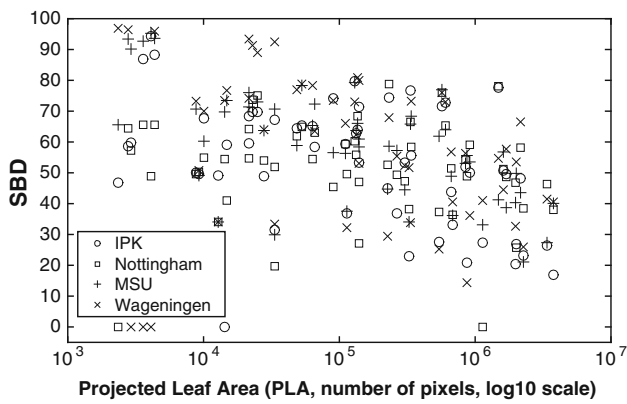


Fig. 13 Effect of plant size, measured as number of plant pixels in ground truth, (projected leaf area) on leaf segmentation accuracy, i.e. SBD, for ‘A3’. Each method is marker coded separately

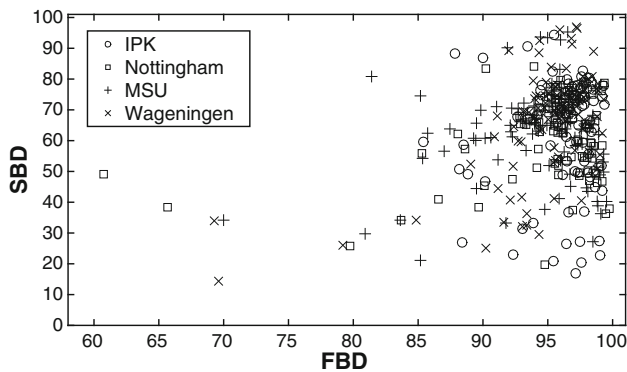


Fig. 14 Effect of plant versus leaf segmentation accuracy (FBD vs. SBD). Each method is marker coded separately. Data from all datasets pooled together; results with FBD < 60% are omitted for clarity

performance, but the inherent assumption of low overlap that each method relies on to find leaves. As a result, most approaches miss small leaves and sometimes miscount other plant parts for leaves. The Wageningen algorithm is more resilient to this problem, presumably due to the optimization of the basins threshold. When the threshold increases, the leaf count decreases. The thresholds were tuned with respect to the best SBD, but apparently this also affects DiC. A positive effect is also due to emphasizing spaces between leaves and stems, avoiding the problem of small spaces between leaves being wrongly segmented as foreground, resulting in a higher number of leaves.

7.5 Performance under blinded shape-based metrics

Most of the metrics we adopted for the challenge rely on segmentation- and area-based measurements (cf. Sect. 3). It is thus of interest to see how the methods perform on metrics that evaluate boundary accuracy and best preserve leaf shape. Notice that these metrics were not available to the participants (hence the term blinded), so methods have not been optimized

Table 4 Segmentation and counting results on the training set *assuming foreground segmentation known*

	SBD (%)	DiC	DiC
IPK			
A1	79.1 (5.5)	2.1 (1.4)	−1.9 (1.7)
A2	80.7 (10.8)	1.2 (1.3)	−1.1 (1.4)
A3	71.0 (20.6)	1.8 (1.8)	−1.8 (1.8)
ALL	78.2 (10.4)	1.9 (1.5)	−1.8 (1.7)
Nottingham			
A1	71.0 (7.2)	4.4 (1.7)	−4.4 (1.7)
A2	66.5 (21.6)	2.5 (1.5)	−2.7 (1.5)
A3	59.5 (11.3)	2.4 (1.3)	−2.4 (1.3)
ALL	68.6 (12.1)	3.9 (1.9)	−3.9 (1.9)
MSU			
A1	78.5 (5.5)	2.5 (1.4)	−2.5 (1.4)
A2	77.4 (8.1)	1.6 (1.3)	−0.9 (1.9)
A3	76.1 (14.1)	1.2 (1.2)	−1.1 (1.2)
ALL	78.0 (7.8)	2.2 (1.4)	−2.0 (1.6)
Wageningen			
A1	77.3 (4.9)	1.5 (1.3)	−0.3 (2.0)
A2	75.5 (8.0)	1.3 (1.3)	−0.9 (1.6)
A3	76.5 (14.6)	1.4 (1.3)	−1.3 (1.4)
ALL	76.9 (7.6)	1.5 (1.3)	−0.5 (1.9)

Shorthands and abbreviations as in Table 2

The best results for each metric are denoted in bold

for such metrics. For brevity, we present results on the testing set only.

We adopt two metrics based, respectively, on the Modified Hausdorff Distance (MHD) [19] and Pratt’s Figure of Merit (FoM) [43], to compare point sets \mathcal{A} and \mathcal{B} denoting leaf object boundaries.

The Modified Hausdorff Distance (MHD) [19] measures the displacement of object boundaries as the average of all the distances from a point in \mathcal{A} to the closest point in \mathcal{B} . With

$$D(\mathcal{A}, \mathcal{B}) = \frac{1}{|\mathcal{A}|} \sum_{p \in \mathcal{A}} \min_{q \in \mathcal{B}} \|p - q\|, \quad (5)$$

where $\|\cdot\|$ is the Euclidean distance, MHD is defined as:

$$\text{MHD}(\mathcal{A}, \mathcal{B}) = \max \{D(\mathcal{A}, \mathcal{B}), D(\mathcal{B}, \mathcal{A})\}. \quad (6)$$

This metric is known to be suitable for comparing template shapes with targets [19]. It prioritizes leaf boundary accuracy, being relevant for shape-based leaf recognition purposes.

Pratt’s Figure of Merit (FoM) [43] was introduced in the context of edge detection and penalizes missing or displaced points between actual (\mathcal{A}) and ideal (\mathcal{I}) boundaries:

$$\text{FoM}(\mathcal{A}, \mathcal{J}) = \frac{1}{\max\{|\mathcal{A}|, |\mathcal{J}|\}} \sum_{i=1}^{|\mathcal{A}|} \frac{1}{1 + \alpha d_i^2}, \quad (7)$$

where $\alpha = 1/9$ is a scaling constant penalizing boundary offset, and d_i is the distance between an actual boundary point and the nearest ideal boundary point.

Let B^{ar} and B^{gt} be sets of leaf object boundaries extracted from leaf segmentation masks L^{ar} and L^{gt} , respectively, where B^{ar} is the algorithmic result and B^{gt} is the ground truth. To evaluate how well leaf object shape and boundaries are preserved, and to follow the spirit of SBD defined in Sect. 3, we use:

- Symmetric Best Hausdorff (SBH), the symmetric average MHD among all object (leaf) boundaries, where for each input label the ground truth label yielding minimum MHD is used for averaging. Best Hausdorff (BH) is defined as

$$\text{BH}(B^a, B^b) = \begin{cases} \sqrt{w^2 + h^2} & \text{if either } B^a = \emptyset \text{ or } B^b = \emptyset \\ \frac{1}{M} \sum_{i=1}^M \min_{1 \leq j \leq N} \text{MHD}(B_i^a, B_j^b) & \text{otherwise} \end{cases} \quad (8)$$

where B_i^a for $1 \leq i \leq M$ and B_j^b for $1 \leq j \leq N$ are point sets corresponding to the boundaries, respectively, B^a and B^b , of leaf object segments belonging to leaf segmentations L^a and L^b ; w and h denote, respectively, width and height of the image containing the leaf object. SBH is then:

$$\text{SBH}(B^{\text{ar}}, B^{\text{gt}}) = \max \{ \text{BH}(B^{\text{ar}}, B^{\text{gt}}), \text{BH}(B^{\text{gt}}, B^{\text{ar}}) \}. \quad (9)$$

SBH is expressed in units of length (e.g. pixels or millimetres) and is 0 for perfectly matching boundaries. If B^{ar} is empty, SBH is equal to the image diagonal (i.e. the greatest possible distance between any two points).

- Best Figure of Merit (BFoM), the average FoM among all leaf objects, where for each input label the ground truth label yielding maximum FoM is used for averaging.

$$\text{BFoM}(B^{\text{ar}}, B^{\text{gt}}) = \frac{1}{M} \sum_{i=1}^M \max_{1 \leq j \leq N} \text{FoM}(B_i^{\text{ar}}, B_j^{\text{gt}}), \quad (10)$$

We express BFoM as a percentage, where 100 % denotes a perfect match.

Table 5 Segmentation results on the testing set with respect to leaf shape

	SBH (pix)	SBH (mm)	BFoM (%)
IPK			
A1	9.2 (3.2)	1.54 (0.53)	62.6 (7.3)
A2	6.9 (3.6)	1.15 (0.60)	66.9 (8.1)
A3	174.9 (442.8)	7.00 (17.7)	41.9 (17.3)
ALL	103.6 (343.5)	4.62 (13.4)	51.1 (17.6)
Nottingham			
A1	13.0 (5.9)	2.17 (0.99)	58.7 (9.0)
A2	9.3 (5.7)	1.55 (0.95)	62.3 (7.4)
A3	193.6 (589.7)	7.74 (23.6)	49.2 (21.8)
ALL	115.8 (453.1)	5.30 (17.8)	53.6 (18.1)
MSU			
A1	13.3 (5.6)	2.22 (0.94)	50.9 (10.3)
A2	10.0 (6.3)	1.67 (1.05)	52.0 (12.2)
A3	81.1 (105.6)	3.24 (4.22)	46.5 (19.0)
ALL	51.7 (86.6)	2.76 (3.25)	48.5 (16.1)
Wageningen			
A1	13.0 (5.6)	2.17 (0.94)	54.1 (9.1)
A2	10.2 (7.8)	1.70 (1.30)	61.1 (9.7)
A3	109.1 (227.0)	4.36 (9.08)	43.7 (26.7)
ALL	67.7 (177.6)	3.38 (6.90)	48.8 (21.8)

Shorthands and abbreviations as in Table 2. Notice that for SBH lower is better, whereas for BFoM higher is better

The best results for each metric are denoted in bold

In Table 5, we see the results on the testing set using these metrics. SBH values vary widely between dataset ‘A3’ and the others (‘A1’ and ‘A2’). This indicates an issue when using SBH with images of different size. Being a distance, SBH given in pixels is dependent on resolution. We therefore also provide this in real-world units i.e. mm, even though object resolution depends on (the non-constant) local object distance from the camera.

Overall, MSU reports best average performance according to SBH (although this result is largely influenced by the ‘A3’ dataset) with IPK performing best on ‘A1’ and ‘A2’. The good performance of MSU does not come unexpected, as optimizing the Chamfer Matching distance boils down to minimizing $D(\mathcal{A}, \mathcal{B})$ from Eq. (5), leading to SBH.

With respect to BFoM, IPK again performs best on ‘A1’ and ‘A2’, while Nottingham outperforms the other methods on ‘A3’. Interestingly, the overall ranking of the methods according to the two metrics is opposite.

MSU exhibits lower variance compared to IPK, Nottingham, and Wageningen, since the latter methods include some empty segmentations (i.e. no leaf objects found) in the testing results, which in BFoM evaluates to 0, and in SBH to the image diagonal length. This situation occurs for some images of very small plants, which are probably missed in the plant segmentation steps of the methods.

7.6 Differences among datasets

Although the tobacco dataset, ‘A3’, has higher resolution and leaf boundaries are more evident, rich shape variation and large overlap among leaves challenge all methods: almost all achieve lower performance compared to ‘A1’ and ‘A2’ (Tables 2 and 3). Even the variability in accuracy increases for ‘A3’. The MSU algorithm shows the least variability among datasets probably due to the fact that it uses templates (rotated and scaled). As such it can adapt better to different shape variability and heavier occlusions and is more robust to plant segmentation errors. It is also due to the reliance on an edge map to fit the templates: on ‘A3’ it can be estimated more reliably compared to ‘A1’ and ‘A2’, where some images can be blurry (due to larger field of view) and resolution is lower. However, when foreground is known (Table 4), variability of the Wageningen solution also becomes lower between datasets.

While ‘A2’ does contain images from different mutants, it shows a different image background with respect to ‘A1’ (black textured tray vs. red smoother tray). When a plant mask is known, SBD results on the training set show (Table 4) that Nottingham, MSU, and Wageningen still do better in ‘A1’ than in ‘A2’, and all methods show higher variances in ‘A2’ than in ‘A1’. So it might appear that different mutants play a role; however, this result is not conclusive since ‘A2’ has fewer images than ‘A1’. In fact, a simple unpaired t-test between SBD in ‘A1’ and ‘A2’ shows no statistical difference (for any of the methods).

We should point out that both Nottingham and Wageningen use the same mechanism to segment leaves: a watershed on the distance (from the boundary) map. However, Nottingham relies on finding first the centres and then using those as seeds for leaf segmentation, while Wageningen obtains an over-segmentation and then merges parts using a threshold on the basins. Their performance difference due to this algorithm selection becomes apparent when comparing results with given foreground segmentation (Table 2). We see that the Wageningen algorithm does better compared to the Nottingham solution. We conclude that finding suitable seeds for segmentation is hard and further, comparing Fig. 10, that this is true especially for small leaves. On the other hand, it appears that the Wageningen algorithm finds a suitable threshold for merging according to the dataset.

7.7 Discussions on experimental work

Through this study, we find that plant segmentation can be achieved with unsupervised approaches reaching average accuracy above 90%. As we suspected, whenever complications in the background are present, they do lower plant segmentation accuracy (explaining also the large variation in performance). Possibly higher performance (and lower

variability) can be obtained with methods relying on learned classifiers and active contour models [34]. Lower plant segmentation accuracy negatively affects leaf segmentation accuracy in almost all cases. Nevertheless, a first level measurement of plant growth (as PLA) can be achieved with a relatively good accuracy, although methods that obtain high average and low variance should be sought-after.

On the other hand, measuring individual leaf growth on the basis of leaf segmentations shows currently low accuracy. The algorithms presented here show an average accuracy of 62.0% (best 63.8%, see Table 3) in segmenting a leaf and almost always miss some leaves, particularly under heavy occlusions when both small (young) and larger (mature) leaves are present within the same plant. SBD does not necessarily capture that, but it is evident when analysing leaf size vs. Dice accuracy and leaf counts. On several occasions, leaf count is not accurate (missing several leaves), and frequently the algorithms are wrongly labelling disconnected leaf parts (particularly their stems) as leaves.¹⁰

Several approaches (IPK and Nottingham) assume that once a centre of a leaf is found that segmentation can be obtained by region growing methods. Naturally, when leaves heavily overlap they do fail to identify the centres (and find less leaf centres than in the ground truth), which holds for both rosette plants considered here. Also when image contrast is not ideal, lack of discernible edges leads to a mis-estimation of leaf boundaries. This is particularly evident in the Arabidopsis data (‘A1’ and ‘A2’) and affects approaches that rely on edge information (MSU). The tobacco dataset (‘A3’), being high resolution, does offer superior contrast, but the amount of overlap and shape variation is significant leading to under-performance for most of the algorithms.

We also investigate performance on leaf boundaries using SBH and BFoM (cf. Sect. 7.5). SBH penalizes boundary regions being far away from where they should be, while BFoM acknowledges boundaries being in the right position. Thus, from a shape-sensitive viewpoint, low SBH is needed if boundary outliers lead to low performance, whereas high BFoM is advisable if an algorithm is robust against boundary outliers. When choosing from the solutions presented here, a trade-off needs to be found, as high BFoM (good) comes with high SBH (bad) and vice versa.

Evident by the meta-analysis of all results is the effect of plant complexity (due to plant age, mutant, or treatment) on algorithmic accuracy. Leaf segmentation accuracy decreases with larger leaf count (Fig. 12), using leaf count as a proxy for maturity [15]. This is expected: as the plant grows and becomes more complex, more leaves and higher overlap between young and mature leaves are present. Overall, most

¹⁰ This indicates that additional (possibly tailored) evaluation metrics may be necessary, although our testing with some common in the literature did not yield any improvement.

methods face greater difficulties in detecting and segmenting smaller (younger) leaves (Fig. 10), most likely not due to their size, but overlap: they tend to grow on top of more mature leaves.

Moving forward, no approach here relies on learning a model on the basis of the training data to obtain leaf segmentations and this might lead to promising algorithms in the future. Interestingly, some of our findings on learning to count leaves do show that leaf count can be estimated without segmentation [21]. However, individual and accurate leaf segmentation is still important: for example, studying individual leaf growth, tracking leaf position and orientation, classifying young from old leaves, and others.

One alternative which changes the problem definition and may reduce complexity is to provide additional data such as temporal (time-lapse images) and/or depth (stereo and multi-view imagery) information. The former can be used for better leaf segmentation, e.g. via joint segmentation and tracking approaches [59,60]. Both types of information will help in resolving occlusions and obtaining better boundaries. Such data are publicly available in the form of a curated dataset [35], and a software tool was released to facilitate leaf annotation [36].

8 Conclusion and outlook

This paper presents a collation study of a variety of leaf segmentation algorithms as tested within the confines of a common dataset and a true scientific challenge: the Leaf Segmentation Challenge of CVPPP 2014. This is the first of such challenges in the context of plant phenotyping and we believe that such formats will help advance the state of the art of this societally important application of machine vision.

Having annotated data in the public domain is extremely beneficial and this is one of the greatest outcomes of this work. The data can be used not only to motivate and enlist interest from other communities but also to support future challenges (similar to this one). We all believe that here is the future: it is via such challenges that the state of the art advances rapidly and a new challenge for 2015 has already been held.¹¹ However, these challenges should happen in a rolling fashion, year-round, with leader boards and automated evaluation systems. It is for this reason that we are considering a web-based system, e.g. similar in concept to Codalab,¹² for people to submit results but also deposit new annotated datasets. This has been proven useful in other areas of computer vision (consider for example PASCAL VOC [20]) and it will benefit also plant phenotyping.

In summary, the better we can “see” plant organs such as leaves via new computer vision algorithms, evaluated on common datasets and collectively presented, the better quality phenotyping we can do and the higher the societal impact.

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Author contributions SAT coordinated this collation study. SAT and HS organized the original LSC challenge. SAT, HS, and MM, wrote the paper and performed analysis. All other authors have contributed methods, text, and results. All authors have approved the manuscript.

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¹¹ See the new Leaf Counting Challenge of CVPPP 2015 at BMVC (<http://www.plant-phenotyping.org/CVPPP2015>).

¹² <https://www.codalab.org/>.

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Hanno Scharr received his Doctoral degree in Physics from Ruprecht-Karls University, Heidelberg, Germany in 2000 and Habilitation in Computer Science from Goethe University, Frankfurt, Germany in 2014. From 2002 to 2003 he was Senior Scientist at Intel, Santa Clara, California. Currently he is working as a Senior Scientist at IBG-2: Plant Sciences, Forschungszentrum Jülich, Jülich, Germany. His areas of interest include computer vision and

image processing, robotics and automation for applications in plant sciences.



Massimo Minervini received the B.Sc. and M.Sc. degrees in Computer Science from the University of Bari, Italy, in 2008 and 2010, respectively. In 2015 he received the Ph.D. degree in Computer Science and Engineering from IMT Institute for Advanced Studies, Lucca, Italy, investigating application-aware image compression for plant phenotyping. He is currently a post-doctoral researcher at IMT Lucca. His research interests include data compression, com-

puter vision, machine learning, and applications to life science.



Andrew P. French received his Ph.D. in Computer Science in 2005 from the University of Nottingham (UK), specialising in computer vision tracking algorithms. He is a member of the schools of Computer Science and Biosciences at the University of Nottingham, where he is part of the Computer Vision Lab and the Centre for Plant Integrative Biology. His research interests include developing image analysis for plant phenotyping in the lab and field.



Christian Klukas received his diploma certificate in Business Computing in 2003 from Otto-von-Guericke University, Magdeburg, Germany, and his Ph.D. certificate in 2009 from Martin-Luther-University, Halle (Saale), Germany. From 2010 to 2015 he led the Image Analysis group at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany. As lead of the Data Analytics group at LemnaTec GmbH, Aachen, Germany,

he works on the topics of high-throughput image analysis, machine learning and data post-processing.



David M. Kramer received Ph.D. in Biophysics in 1990 from U. Illinois at Urbana-Champaign, USA. He was then post-doctoral research associate, first at U. of Illinois, then at the Institut de Biologie Physico-Chimique, Paris. In 1995, he became a faculty member at the Institute for Biological Chemistry at Washington State University. In 2010 he was recruited as the Hannah Distinguished Professor in Photosynthesis and Bioenergetics at the MSU-DOE

Plant Research Lab at Michigan State University, USA. His work on photosynthetic phenotyping led to the establishment of MSU's Center for Advanced Algal and Plant Phenotyping (CAAPP), a multi-disciplinary and multi-user facility to phenotype photosynthetic processes. He is co-founder of Phenometrics, Inc., a spin-off company from MSU, which manufactures advanced photobioreactors for optimizing algal bioenergy production. He also co-founded the PhotosynQ.org project, a network of scientists, researchers, extension agents, sophisticated scientific instruments and analytical tools that facilitates plant phenotyping around the world.



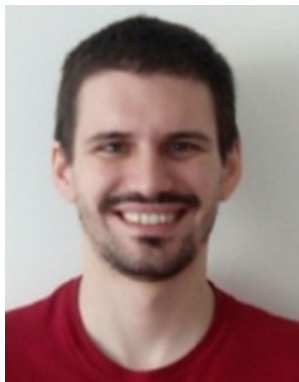
Xiaoming Liu is an Assistant Professor in the Department of Computer Science and Engineering at Michigan State University (MSU). He received the M.E. degree in Computer Science from Zhejiang University, China in 2000, and the Ph.D. degree in Electrical and Computer Engineering from Carnegie Mellon University in 2004. Before joining MSU in Fall 2012, he was a research scientist at General Electric Global Research Center. His research areas are biometrics,

image alignment, video surveillance, computer vision and pattern recognition. He has authored more than 90 scientific publications, and has filed 22 U.S. patents.



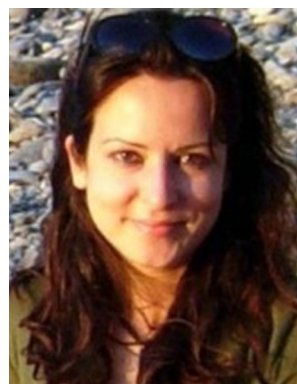
Gerrit Polder is researcher Computer Vision and Plant Phenotyping at the department Greenhouse Horticulture of Wageningen University and Research Center. In 1985 he obtained a B.Sc. in electronics at the HAN University of Applied Sciences in Arnhem the Netherlands. After working several years in image processing and related topics, in 2004 he got a Ph.D. from Delft University of Technology on Spectral imaging for measuring biochemicals in

plant material. From 2004 he works on machine-vision and phenotyping projects. His research interests are mainly on hyperspectral and multispectral imaging for disease detection, 3D imaging using stereo-vision, Time Of Flight Imaging, and light-field technology and sensor fusion (color, fluorescence and infrared) for monitoring plant health.



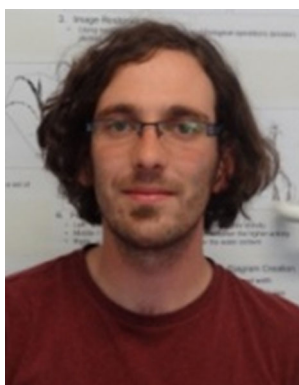
Imanol Luengo received his B.S. in Computer Science and his M.S. (by research) in Computational Engineering and Intelligent Systems, both from the University of the Basque Country, Spain in 2012 and 2013 respectively. He is now pursuing his Ph.D. (since 2014) from the University of Nottingham in the area of image processing methods for biomedical volume analysis. His areas of interest include low level vision tasks for data preprocessing and high level vision tasks,

such as segmentation and semantic labelling with superpixels.



Danijela Vukadinovic received her M.Sc. degree in Telecommunications and Image Processing from the University of Belgrade, Serbia in 2003. She was a researcher in TU Delft, the Netherlands, in the field of Facial Emotion Recognition in the 2004-2006 period. She received her Ph.D. degree in Biomedical Image Processing from the University of Rotterdam, the Netherlands, in 2012. Currently, she is focused on horticulture topics being a memeber of the

Greenhouse Technology Group, Wageningegn University and Research Center, the Netherlands. Her research interests include Computer Vision, Machine Learning, Robotics and Phenotyping.



Jean-Michel Pape received his B.S. in Computational Visualisitics from the Otto-von-Guericke University in 2012. He is currently working in the research group Image Analysis at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK). His research is focused on image processing, computer vision and machine learning in the context of high-throughput plant phenotyping.



Xi Yin received the B.S. degree in Electronic and Information Science from Wuhan University, China, in 2013. Since August 2013, she has been working toward her Ph.D. degree in the Department of Computer Science and Engineering, Michigan State University, USA. Her research interests include image processing, pattern recognition, and computer vision.



Sotirios A. Tsafaris received the M.Sc. and Ph.D. degrees in Electrical and Computer Engineering from Northwestern University (USA), in 2003 and 2006, respectively, and the Diploma degree in Electrical and Computer Engineering from the Aristotle University of Thessaloniki (Greece) in 2000. He is currently a Chancellor's Fellow (at a Senior Lecturer level) at the University of Edinburgh (UK). Previously he was an Assistant Professor with IMT Institute for

Advanced Studies, Lucca (Italy). He remains as the Director of the Pattern Recognition and Image Analysis Unit at IMT. Prior to that, he held a joint Research Assistant Professor appointment at Northwestern University with the Departments of Electrical Engineering and Computer Science (EECS) and Radiology Feinberg School of Medicine. He maintains an adjunct appointment with EECS. He has published extensively, particularly in interdisciplinary fields, with more than 100 journal and conference papers in his active record. His research interests are image analysis, image processing, data mining and machine learning, and distributed computing. He is considered one of the pioneers in affordable image-based phenotyping, and recently with colleagues has developed the Phenotiki platform. He serves the academic community regularly, and in phenotyping he has co-organized twice the Computer Vision Problems in Plant Phenotyping (CVPPP) once at ECCV 2014 and at BMVC 2015.